ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

DEPARTMENT OF ZOOLOGY

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CERTIFICATE I

This is to certify that the thesis entitled **"In-vitro evaluation of acaricidal properties of *Cymbopogon citrates* and *Nicotiana tabacum* against *Rhipicephalus spp.* collected from cattle and pet dogs"** submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology to the Orissa University of Agriculture and Technology is a faithful record of bonafied and original research work carried out by Miss Tanushree Moharana under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

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ADVISORY COMMITTEE

CERTIFICATE II

This is to certify that the thesis entitled **"In-vitro evaluation of acaricidal properties of *Cymbopogon citrates* and *Nicotiana tabacum* against *Rhipicephalus spp*. collected from cattle and pet dogs"** submitted by Miss Tanushree Moharana to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the degree of Master of Science in Zoology has been approved by the student's advisory committee and the external examiner.

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(Tanushree Moharana)

CONTENTS

LIST OF TABLES

SL. NO. **PARTICULARS**

LIST OF FIGURES

LIST OF ABBREVIATIONS

TBD	Tick Borne Diseases
AET	Adult Emulsion Test
ITM	Integrated Tick Management
HS	Habitat Suitability
AVHRR	Advanced Very High Resolution Radiometer
gm	Gram
hrs	Hours
ml	Milliliter
L	Liter
µl	Micro-liter
%	Percentage
cm	Centimeter

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ABSTRACT

Tick infestation is one of the reasons for economic losses in dairy farms as well as in pet dogs. Further, development of resistance, high cost of available drugs and hazards associated with the use of chemical acaricides have created interest to evaluate the effects of lemon grass oil, tobacco leaves and compare their effectiveness with deltamethrin against *Rhipicephalus spp.* The female ticks were particularly targeted by these acaricides as they are directly responsible for propagation of the parasites. Evaluation of acaricidal property was performed using different concentrations of the lemon grass oil, tobacco leaves, deltamethrin and the study was conducted by following the method of adult emulsion test (AET). Through the present study, it was found that lemon grass oil has better acaricidal property than the tobacco leaves. Though deltamethrin had the same effect, it had negative impact on the animal as well as on the ecosystem. So, lemon grass oil, a phyto-remedy, is suggested as a better alternative to cure the tick infestations. Further studies on pharmacologically active ingredients of lemon grass oil are necessary to replace its competitors.

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DISCUSSION

Ticks are one of the most harmful parasites affecting bovines, pet dogs and many other animals like goat, sheep, buffalo, horse etc. These ticks acts as vectors of several diseases like anaplasmosis, babesiosis, ehrlichiosis and tick infestation leads to loss of milk production in cattle, loss of body weight, loss of body hair, debility, stunted growth, anemia in both cattle and dogs. They also cause severe toxic conditions leading to paralysis, irritation and allergy. The most common combined effect of TBDs in Indian dairy system is reduction in milk yield i.e. loss of 14% of the lactation and quality of hides for leather industry (Biswas, 2003). Periodic administration of acaricides is preferred managemental practices to bring down the tick population and to bring it in control. But application of such preparations is not only expensive but also considered hazardous at times (FAO, 1995). On the other hand, survey of literature revealed potentiality of plants against various pathogens. With these backdrops, the present study was designed to compare in vitro effects of frequently used chemical acaricide and two commonly available plants extracts on ticks.

Rhipicephalus spp. is a host tick; it's all stages are spent on one animal. The eggs hatch on the ground, on grass or below the rocks and the larvae crawl up the grass or the other plants to find a host. In summer, it can survive for as long as 3-4 months without feeding. In cooler temperature, they may live without food for up to 6 months. Tick that does not find a host eventually die of starvation. Newly attached seed ticks (larvae) usually found on the softer skin inside the thigh, flanks forelegs and brisket. After feeding larvae moult twice to become nymphs and then adults. Each developmental stage (larvae, nymph and adult) feeds only once, but the feeding takes place over several days. Adult male ticks become sexually mature after feeding and mate with feeding females. An adult female tick after mating and feeding detaches from the host and deposits a single batch of eggs in crevices or derbies or under stones. The female tick dies after ovipositing. As single life cycle is completed in about 60 days and 4 generations can been recorded in one year.

Blood is the only form of nutrient taken by ticks from the hosts and large blood meals are required for their development and survival (Ribeiro, 1995). Ticks are pool feeders that accomplish feeding by lacerating small blood vessels and sucking up the blood that flows to the wound, the so-called feeding site (Mans, 2011). Though both the males and females feed blood, the control of female ticks is more essential as the feeding of higher temperature blood of cattle and dogs (mammals) helps in the maturation of eggs. A single female lays about 2000 or more eggs in clusters and eggs hatch into 6 celled larvae after 2-6 weeks. Hence, the females are more dangerous than males and their control is more important. So, it is necessary to distinguish male ticks from female ticks. The ticks collected were separated into males and females basing on the presence of the ovipositor. The females possess a smaller size flexible scutum limited to a small region directly

behind the head (capitulum), softer cuticle and larger body size. Males have adanal shields and accessory shields. The anal groove is absent or indistinct in females and faint in males. Cattle ticks have a hexagonal basis capitulum. The spiracular plate is rounded or oval and the palps are very short, compressed and ridged dorsally as well as laterally. Female cattle ticks attend a length of 0.5 mm – 100mm and when fully engorged, body appears larger than normal size. It is bean seed shaped with hard and wrinkled skin, olive green to bluish gray in colour and has four pairs of appendages. The males are similar in structure but lack the ovipositor. The dog ticks are egg shaped and fully engorged ticks are slightly larger in size. They are brownish red when non-engorged. They are 0.5mm in length and have four pairs of clearly marked jointed appendages. Cattle and Dog ticks i.e. *Rhipicephalus spp.* belong to Phylum: Arthropoda, Class: Arachnida, Order: Acarina, Family: Ixodidae.

Available literatures on the use of medicinal plants were reviewed and discussions were held with many persons associated with ayurvedic preparations regarding their use on the ticks. It was found that many plants possess acaricidal properties. However, due to easy availability *Cymbopogon citrates* (lemon grass oil) (Heimerdinger, 2006) and *Nicotiana tabacum* (tobacco leaves) (Tylor, 1994) have been selected for the present study. In addition, a commonly used chemical acaricide deltamethrin was included for comparison of in vitro efficacy on ticks. *Rhipicephalus spp.* of ticks infesting cattle collected from rural and urban areas and also from different pet dogs were exposed these chemicals.

Deltamethrin is a widely used acaricide that acts at a concentration of 3ml/L as per clinically recommended dosages. It has been observed that at this concentration, it was only 20% effective within 1 hour of exposure and 97% effective within 24 hours of exposure on cattle ticks collected from urban areas and not effective on ticks collected from different dairy farms located in rural areas. Its frequent application and unawareness of the dairy managers have made the ticks to become unresponsive against this concentration. Hence, its concentration was raised to 6ml/ L of water but the effect was not so good. Such unresponsiveness was also seen in case of dog ticks at a concentration of 5ml/L of water. Jyothimol in 2014 showed that due to low level, resistance was developed in the most economically important cattle infesting ticks and it cautions the development of large scale resistance in future. Adult immersion test (AIT) and larval packet test (LPT) were optimized using laboratory reared susceptible line of *Rhipicephalus microplus* (IVRI-I) for determination of 95% lethal concentration (LC_{95}) of deltamethrin (29.6 ppm in AIT and 35.5 ppm in LPT) (Anil et al., 2012). Deltamethrin not only possess a threat to the animal toxicity but also indirectly to the environment. Yufang *et al.* (2015) showed the long term toxic effect of deltamethrin in soil at 602.15µg/cm^2 concentration. When deltamethrin mixes with waterbodies, it acts as a pollutant causing acute intoxication in monosex Nile tilapia (*Oreochromis niloticus)* (Sayed

et al., 2007), on the fry rainbow trout (*Oncorhynchus mykiss*) (Ural *et al.*, 2005), on Indian major craps *Labeo rohita* at LC_{50} value of 0.438 mg/L (Lenin *et al.*,2015), also affects swimming velocity and biomarkers of the common prawn *Palaemon serratus* (Cristiana *et al.*, 2012). Hence, its use must be avoided and replaced with some nontoxicant, ecofriendly, nonharmful herbal products.

Lemon grass (genus *Cymbopogon*) is a herb of family Poaceae, whose main constituentis citral (3,7-dimethyl-2,6-octadien-1-al) (Abe *et al*.,2003). Lemongrass oil has been found to contain up to 75-85% citral (Nhu-Trang *et al*., 2006). Lemon grass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonene, piperitone, citronellal, carene-2, alpha-terpineole, pinene, farnesol, proximadiol, and (+)-cymbodiacetal (D'Souza *et al*.,2004). Lemon grass oil has been described to have various medicinal properties and possess a wide spectrum of antibacterial and antiviral activities.

A test was conducted to assess the effect of *Cymbopogon citratus* on levels of serum aflatoxin-albumin (AF-albumin) adducts following aflatoxin B (1) {(AFB (1)} exposure in rats. Rats were treated with 5g/kg *Cymbopogon citratus* extract daily for one week, then the rats were administered AFB(1). No significant difference in biomarker levels was seen with the *Cymbopogon citratus* extract treatments compared to control rats (Vinitketkumnuen *et al*., 1999). In present experiment, this oil was found to be more than 80% effective at a concentration of 0.5% and 1% within 1hr and 24 hrs of exposure. Batish (2008) used eucalyptus oil as a natural pesticide. 56% and 62% tick mortality was observed after 24 hours of deeping at a concentration of 0.5% and 1% (Chungsamarnyart *et al*., 2001). Hence, lemon grass oil can be used as an acaricide instead of eucalyptus as it has a better effect.

A number of pharmacological activities of tobacco leaves have also been reported. The plant belongs to the genus *Nicotiana* of family Solanaceae (night shade). In the tobacco plant, nicotine is synthesized in the roots of the plant and then stored in its leaves. It is a repellent and a powerful neurotoxin for insects. Tobacco is an alkaloid chemical, similar to caffeine. Because of its properties as an alkaloid, nicotine can serve as a stimulant to mammals in small quantities.

Results of this study showed that more than 90% of ticks could not survive in either deltamethrin or lemon grass oil. Contrary, no ticks died in normal water. So, it is concluded that both were equipotent in inhibiting the survival of ticks. Tobacco leaf powder also acted as an acaricide to ticks, but it had less effect than deltamethrin and lemon grass. As deltamethrin has various side effects on the host animals, lemon grass oil may be preferred for use on cattle and pet dogs to inhibit the tick infestation.

Reduction of tick infestation in cattle and dogs could be targeted through breaking down the process life cycle at oviposition stage. In other words, inhibition of female ticks on the hosts before egg laying is reasonable attempts to disrupt continuity of life cycle. The present work revealed the capacity of lemon grass oil as a potential alternative to chemical acaricides. Undoubtedly, it is a novel approach, befitting to modern concept. However, further studies may be undertaken to throw more light on the effects of pharmacologically active components.

INTRODUCTION

Ticks are the one of the most important ectoparasitic pests that requires blood meal to complete their life cycle. The *Rhipicephalus spp.* is considered to be the most harmful parasites on cattle and dog in sub-tropical areas of the world (Evans *et al.*, 2000). Ticks create a major problem in domesticated cattle and dogs in tropical and subtropical countries (Jongejan and Uilenberg, 1994). They act as vectors of several diseases and cause major problem to livestock health in the world. The economic losses due to tick parasitism are direct losses affecting hosts and indirect losses via transmission of tick borne disease causing pathogens (Jonsson, 2006, Reck *et al.*, 2013). The severity of tick borne diseases (TBDs) depends on geographic region, type species, host population, socioeconomic factors and technological advances in control measures (Morelos, 1991). It has been estimated that the global loss due to tick infection has been US $14000-18000 million annually and the cost of management of TBDs in live stock of India is as high as US $498.7 million per annum (Minijauw and McLeod, 2003). Ticks rank first among arthropod vectors of disease causing protozoa, bacteria and viruses in nonhuman vertebrates and rank second only to mosquitoes as vectors of pathogens to human (Zhou *et al.*, 2009). These ticks bite the hosts (cattle and dogs) causing irritation to skin, allergic reactions, anemic condition, hair loss dermatitis and give poor aesthetic value to the hosts. The ticks are vectors of babesiosis and anaplasmosis that cause fever in cattle and dogs. Biophysical factors, predominantly the season of initiations had largest influences on tick population, followed by habitat type and level of initial infestation (Teet *et al*., 2003). Tick and TBD control are mainly focused on wide spread use of various acaricides like organophosphates, carbonates, pyrethroids, cyclodines, amidines, macrocyclic lactones. Their use leads to various problems such as drug resistance, residual deposition, environmental pollution and high cost of treatment. In India, the TBDs are also controlled by intensive use of chemical acaricides. However, the ticks develop resistance to these chemicals at a speed, similar to the rate of application of new variety of chemicals. Dependence on the use of insecticides to control *Heamatobia irritans* in Brazil has resulted in the development of resistance, a concomitant increase in production cost, risk to human and environmental health and food contamination (Barros, 2004).

As tropical and sub tropical climate is suitable for ticks to complete their life cycle, they are more prevalent in these areas, especially in Odisha. Odisha lying just south of the Tropic of Cancer, has a tropical climate and the weather is also greatly influenced by the sea. The average south-west monsoon rainfall in the state during July – September is 150cm. These conditions also favor the spreading of tick borne diseases. People of Odisha who are associated with diary are mostly unaware of proper management of cattle health. They give less importance to the cattle cleanliness while grazing and in the farm. Hence, the animals are easily get attacked by the ticks and TBDs. Similarly, the health of the pet dogs has equal importance as many people are associated with them emotionally

as partners of their loneliness. They also have chances of getting attacked by the ticks from the surroundings.

As the traditional use of acaricides on animals is associated with several other problems, integrated management is recommended (Ghosh *et al*., 2007). The concept of integrated tick management (ITM) emphasizing the importance of animal management, ecology, epizootiology and economics and marketing in the formulation of integrated policies for ticks and TBDs, was developed during 20th century (Tatchell, 1992). As there is a growing desire among the general population for natural and environmental friendly approach in order to avoid the hazardous effects of chemicals, it has become necessary to find suitable herbal preparations as alternative control strategies. The plants and their products posses a wide range of biological and pharmacological properties. The people in developing countries still rely on the traditional medicines and thus herbal medicines are currently in demand. Its popularity is also increasing day by day. About 500 plants with their medicinal uses are described in ancient literature. Presently about 25% of pharmaceutical prescriptions in United States contains at least 1 plant derived ingredients. India having the richest biodiversity and heritage of medicinal plants is actually lagging behind in this regard. The ancient residents used very peculiar plant extracts as medicines and there are about 25,000 effective plant based formulations. Plant extracts are being used as preventives, promotional and curative agents. It is estimated that there are about 7,800 medicinal drug manufacturing units in India, which consume about 2,000 tons of herbs annually (Verma and Singh, 2008). The crude ethanolic extracts of areal parts of *Leucas aspera* cause complete failure of eclosion of eggs of *Boophilus annulatus* (Ravindran, 2011). *Jatropha curcas* (Lill) leaf extract acts as a possible alternative for population control of *Boophilu sannulatus* (Juliet *et al*., 2012). Tobacco decoction was highly effective in controlling *Sarcoptic scabiei* infection in rabbits (Vasanti *et al*., 2004). As our lifestyle is getting techno-savvy, we are moving away from nature and have not been able to fully explore the natural store house. Certain European and Oriental countries have successfully using the herbal products since centuries. It is highly important to note here that these products are within common man's reach and knowledge, low cost, widely available, more eco-friendly and more over they have no side effects with effective remedies.

Available literature pertaining to in vitro effects of *Cymbopogon citrates* and *Nicotiana tabacum* is scanty. *Cymbopogon citrates* oil has been shown to have insect repellent effects (Ansari *et al.* 1995) and also is an effective insecticide against various insects like dust mites, termites and ticks (Sigma-Aldrich Plant Profiler). On the other hand *Nicotiana tabacum* contains a higher concentration of nicotine, a powerful neurotoxin to insects. Hence, these were selected to study their acaricidal effects on collected ticks.

Keeping in view of present situation of tick control and future option to find suitable herbal preparations as alternative control strategies the study was conducted with following objectives:

- Identification and collection of female ticks from cattle and dogs.
- Collection and preparation of tobacco leaf extract, lemon grass oil and explore their availability in the market.
- Prepare appropriate concentration of chemical acaricide, Deltamethrin available in the market.
- In vitro evaluation of effects of the lemon grass oil extract and tobacco leaf extract and Deltamethrin on cattle and dog ticks.

REVIEW OF LITERATURE

The ticks cause tick fever, tick induced anemia and are responsible for many haemo-protozoan diseases resulting in great economic loss to livestock, hence, their control measure is important (Zahid *et al.*, 2006). In order to eradicate ticks, we must know their prevalence, pathogenesis, control methods like conventional therapy and efficacy of medicinal plant extracts.

2.1 PREVALENCE AND PATHOGENESIS OF TICK INFESTATION IN CATTLE AND DOGS

Drummond *et al.* (1981) found that about 68% of cattle were infected by *Boophilus micropuls* in most of the areas of Barbados.

Rajagopalan and Sreenivasan (1981) studied role of ticks on occurrence of Kyasanur Forest Disease of Karnataka state in cattle and buffaloes and they found 16 species of ticks belonging to 6 genera parasitize cattle and buffaloes. The population included *B. micropuls* and the vector of the Kyasanur Forest Disease Virus, *Haemaphysalis bispinosa.*

Horak (1982) studied the seasonal prevalence of dog ticks and found that they are more abundant during early summer to late summer and the adults were maximal in number in mid-summer and from late summer to autumn as well as during early spring.

Davey *et al.* (1982) studied the seasonal effect on development and ovipositional capability of *B. microplus* and *B. annulatus* reared on cattle and found that more than 95% of *B. microplus* females removed from the host on the 19^{th} day or later during the summer and autumn are ready to lay eggs.

Ouhelli and Pandey (1982) found mean tick per animal ranged from 6.5 to 36.6 in different regions of Morocco during July, 1980.

Pegram *et al.* (1982) analyzed the data from surveys conducted in 1951, 1972 and 1978-79 and found that the tick numbers are generally low in miniature breeds of cattle in the Yemen Arab Republic.

Miranpuri (1988) informed that Ixodid ticks *Anaplasma marginale* and *Babesia bigemina* are in 6.2% and 2.6% respectively on buffaloes tested in the northeastern states and 14.9% and 4.7% respectively in the northwestern states of India.

Lima *et al.* (2000) found that the Holestein cattle blood had significantly ($P<0.05$) greater infestation of *B microplus* in Minas gerais.

Estrada (2001) studied the distribution and habitat suitability (HS) of the tick *B. microplus* using remote sensing, on the basis of AVHRR (advanced very high resolution radiometer) satellite imagery, together with standard geographical methods (cokriging).

Latha *et al.* (2004) studied the seasonal activity of ticks in sheep and goat population from November to December in the year 2000 in Tamilnadu and found that the infestation was greater during the rainy season.

According to the study conducted by Yakhchali and Hasanzadehzarza in 2004 in villages of Oshnavich Suburb during spring 2001 to spring 2002, Ixodid ticks affect the groin and mammary glands more (52.24% in cattle) than the head, ear and nose (1.69% in cattle).

Chitimia (2005) reported a highly significant correlation between the weight of the engorged females and the weight of eggs ($r=0.922$; $p<0.01$) of *D marginatus*, whose pre-oviposition period lasts for 7-25 days (mean, 15.83 days) and the oviposition period for 44-65 days (mean, 42.25 days) in natural condition of temperature and relative humidity.

Opera *et al.* (2005) surveyed the common ticks infesting cattle and tick borne protozoan parasites in Sokoto municipality, Nigeria. Ticks showed the highest preference of attachment on mammary glands (35.6%) and least for the inner sides of thighs and neck region (1.1%) among female cattle and in males, attachment was highest at inguinal region (66.7%) and least at peri-anal region (13.3%).

Aydin *et al.* (2006) surveyed the prevalence of Ixodid ticks on ruminants during spring to early summer in south eastern Bulgaria and found the nymphs of R*hipicephalus sp.* from late March to April.

Estrada-Peña and Fuente (2014) described the ecology of ticks and epidemiology of tick-borne viral diseases.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging haemorrhagic fever that was first described in rural areas of China by Liu *et al.* (2014)

2.2 CONVENSIONAL THERAPY AND RESISTANCE DEVELOPMENT IN CATTLE TICKS AND DOG TICKS

Endris *et al.* (2000) evaluated two topicallied spot-on products, 65% permetrin and 9.7% fipronil used for canine flea and for repellency against *I. ricinus*, the primary vector of lyme disease in dogs in Europe.

Jernigan *et al.* (2000) conducted seven controlled studies to investigate the efficacy of selamectin against weekly infestation of dogs with *Rhipicephalus sanuineus* and *Dermacentor variabilis.*

Roy *et al.* (2000) studied the effect of preventive (Amitraz 9% w/w) collar against tick infestation in dogs that showed effect within 24 hours.

Sarathchandran and Murty (2000) studied the lesions observed in hepatic necrosis and glomerular nephritis caused due to *C. collinus* (karada) poisoning in cattle and buffaloes.

Jons (2001) investigated the efficacy and mode of action of α-cyano-pyrethroid flumethrin against adult hard ticks present on dogs.

Maske *et al.* (2001) conducted an experiment to evaluate the therapeutic efficacy of deltamethrin on ectoparasite infestation in dogs in Nigeria.

Styendra *et al.* (2001) evaluated the therapeutic control of *B. microplus* ticks in cattle and buffaloes with deltamethrin spray.

Sushma *et al.* (2001) studied the efficacy of invermectin and moxidectin in treatment of ectoparasite infestation in dogs.

Fourie *et al.* (2003) compared the efficacy of two sets of collars (Kiltix collar, Bayer Ag), containing different plasticisers and impregnated with acariciodes flumethrin (2.25%) and upoxur (10%) against adult *R. sanguineus* on experimentally infested and kenneled dogs.

Bagherwal (2004) recorded the efficacy of flumethrin against different stages of *Hyalomma anatolicum* ticks.

Silva Vaz *et al.* (2004) reported the effect of several acaricides on the enzyme activity of a *Boophilus microplus* recombinant glutathione S-transferase (rGST).

Costa and Docente (2004) evaluated the efficacy of drug combination ivermectin 2.25% + abamectin 1.25% against endo and ectoparasites of cattle in Brazil.

Arantes *et al.* (2005) experimented on fly and tick killer performance of a chlorpyriphos 12% based on pour-on formulation in naturally infested cattle.

Bagherwal (2005) evaluated the efficacy of cypermethrin at different concentrations against different stages of ticks in vitro by soaked filter paper method.

Campos and Oliveira (2005) conducted in vitro evaluation of resistance and sensitivity of *B. microplus* to most commonly used acaricides such as amidine, deltamethrin, cypermethrin /

dichlorvos and trichlorform compounds / cyfluthrin in the Ilheus region, Brazil during April to September 2000.

The percentage reduction in number of female ticks in cattle treated with ivermectin and moxidectin against *B. microplus* showed 94.8 and 91.1% effectiveness respectively (Davey *et al.*, 2005).

Marra *et al.* (2005) assessed the in vitro and in vivo acaricidal efficacy of Ectophos on natural infestation with *B. microplus.*

Otranto *et al.* (2005) compared the efficacy of the combination of imidacloprid 10%/ permetrin 50% versus fipronil 10%/ s- methoprene 12% against ticks in naturally infested dogs.

Pathan *et al.* (2005) found that butacarboxin (0.25%) was highly effective (95.33%) against *Ixodid* ticks in cattle.

Roy *et al.* (2005) evaluated the efficacy of 1% flumethrin pour-on (PORON, Alembic, Vet-Division, Vadodara) against tick infestation in cattle and its side effects.

Vatsya *et al.* (2006) investigated the efficacy of ivermectin pour-on against one-host tick *B. microplus*, in cattle and buffaloes and its effect on biotic potential of engorged female ticks.

Jonsson *et al.* (2007) evaluated progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick *Boophilus microplus*.

Peer and Ansari (2008) evaluated the clinical and therapeutic management of mange in cattle.

Sharma *et al.* (2008) evaluated the efficacy of amitraz as a topical application and shampoo based cypermethrin and carbaryl (Notix) against tick infestation in pet dogs.

Rosado-Aguilar *et al.* (2008) evaluated the effect of amitraz selection pressure on the development of resistance in field populations of *Boophilus microplus* in the Mexican tropics.

Bissinger *et al.* (2010) reviewed the history and efficacy of tick repellents, discovery of new repellents and areas in need of attention such as assay methodology, repellent formulation and the lack of information about the physiology of repellency.

Ravindran *et al.* (2011) evaluated the toxicity of different solvents, viz. hexane, petroleum ether, n-butanol, isopropyl alcohol, chloroform, glycerol, ethyl acetate, acetone, ethanol, and methanol. The study revealed that methanol was least toxic solvent against *Rhipicephalus* (*Boophilus*) *annulatus*.

Rajakumar and Rahuman (2012) evaluated acaricidal activity of aqueous extract and synthesized silver nanoparticles from *Manilkara zapota* against *Rhipicephalus* (*Boophilus*) *microplus*.

Sharma *et al.* (2012) conducted a cross sectional study to assess the prevalence of synthetic pyrethroids (SP) resistance in *Rhipicephalus (Boophilus) microplus* in India.

Kumar *et al.* (2013) Surveyed of pyrethroids resistance in Indian isolates of *Rhipicephalus* (*Boophilus*) *microplus* and Identified C190A mutation in the domain II of the para-sodium channel gene.

Rodriguez-Vivas *et al.* (2013) evaluated the efficacy of cypermethrin, amitraz, and piperonyl butoxide (PBO) mixtures, through *in vitro* laboratory bioassays and *in vivo* on-animal efficacy trials for the control of resistant *Rhipicephalus* (*Boophilus*) *microplus* on cattle in the Mexican tropics.

Singh *et al.* (2014) made a study to evaluate cypermethrin resistance in *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Muktsar and Mansa districts of Punjab state, India, by using adult immersion test (AIT).

Singh *et al.* (2014) detected resistance levels against cypermethrin and deltamethrin, the most commonly used synthetic pyrethroids (SP), in *Rhipicephalus* (*Boophilus*) *microplus.*

Abbas *et al.* (2014) evaluated acaricide resistance in cattle ticks and approaches to its management.

Perinotto *et al.* (2014) evaluated enzymatic activities and effects of mycovirus infection on the virulence of *Metarhizium anisopliae* in *Rhipicephalus microplus*.

Jyothimol *et al.* (2014) evaluated deltamethrin resistance status in *Rhipicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus* ticks collected from cattle of five organized farms of Kerala, south India.

Nava *et al.* (2015) evaluated the strategic applications of long-acting acaricides for the control of *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina.

Sachin Kumar *et al.* (2015) found that discriminating concentrations of malathion, coumaphos, fenvalerate and fipronil developed acaricide resistance in ticks infesting animals.

2.3 EFFICACY OF MEDICINAL PLANT EXTRACTS ON CATTLE TICKS AND DOG TICKS

Malonza *et al.* (1992) found anti-tick properties of the plant *Gynandropsis gynandra* (L.) brig.

Joshi *et al.* (2000) evaluated herbal madicaments (garlic ointment 15%, neem ointment 5%, himax lotion and himax ointment + teeburb) in sarcoptic mange of rabbits. Topical application of crude garlic extract proved to have distinct miticidal action without any ill effect on the host. Histamine concentration before and after treatment were not significantly different between treatment groups.

Kumar *et al.* (2000) assed the efficacy of herbal ectoparasiticidal compound (AV/EEP/14) (containing the active ingredients; *Cedrus deodara, Pongamia glabra (P. pinnata), Azadirachta indica, Eucalyptus globules and Acorus calamus)* and mixed it with tap water in 1:4 ratio. He sprayed it for five times at six days intervals on cattle and found the cattle completely free of ticks for a period of 30 days after the last treatment.

Lans *et al.* (2000) used medicinal plants for dogs in Trinidad and Tobago

Benavides *et al.* (2001) used the Neem (*Azadirachta indica) extract* as an alternative of cattle tick *B. microplus* control. As a result of adult emulsion test (ALT), highest percentage of control of the reproductive efficacy was observed in ether solution at a 1:5 dilution (100%). The highest reproductive efficacies observed on the alcoholic extracts were 70 and 69% respectively, for dilutions 1:5 and 1:20.

Chungsamarnyart and Jansawan (2001) evaluated the effect of *Tamarindus indicus L.* against *B. microplus.* The crude extract of *Tamarindus indicus* (containing oxalic, malic, succinic, citric and tartaric acid) having oxalic acid acted as a strong acarisidal activity after 24 hour of deeping the ticks at a concentration of 0.5 and 1% (56 and 62% mortality respectively). Tartaric acid at 1% concentration showed highest delayed acarisidal activity (73% mortality of ticks after 7 days of dipping).

Davey *et al.* (2001) evaluated efficacy of a single whole body spray treatment of spinosad (a naturally derived control agent) against *B. microplus* on cattle. Spinosad treatment appeared to be more effective against immature stages (nymphs and larvae) than against adult ticks.

Ponte (2002) evaluated the effectiveness of *manipueira* (a liquid extract from cassava roots) as an acariside in cows (*Bos Taurus)* infested with *B. microplus* and found that manipueira was as effective as the commercial acariside in eliminating tick infestation in cows, with a tick survival rate of nil.

Webb and David (2002) evaluated the use of neem seed kernel 5%(W/V) water extract at a rate of 5gm/kg body weight of animal for controlling common cattle ticks (*Ixodidae).* The tick population densities on animals treated with neem seed extract was lower than the untreated animals.

Kumar *et al.* (2003) evaluated the lousicidal potential of timru (*Zanthoxylum alatum)* on a tropical hen louse (*Lipeurus lawrensis tropicalis)* and it caused 100% mortality within 12 hours.

Srivastav and Das (2003) applied 6 plant oils, namely kaddu (*Cucurbita maxima), Kahua (Terminalia Arjuna), Neem (Azadirachta indica), Karanj (Pongamia pinnata), Ritha (Sapindus trifoliatus) and Castor (Ricinus communis).* Application of oil for 72 hours showed percentage of tick control was highest for ritha oil followed by karanj , neem, kahua, kaddu and castor oil.

Choudhury *et al.* (2004) evaluated the efficacy of aquous extract of *Nicotina tabacum* in undiluted and 50% aqueous diluted form against *R. haemaphasaloides* and found both the forms adversely affected egg laying and production of larvae.

Singh *et al.* (2004) evaluated the acarisidal property of *kuppaimeni (Acalypha indica)* in naturally infested broiler rabbits for its in vitro (48 hour) and in vivo (14 days) activity. The herbal paste had a lethal effect on the livable mites from 4 hour.

Vasanthi *et al.* (2004) found that *Nicotina tabacum* decoction on sarcoptic mange infestation was highly effective in rabbits.

Vatsya and Das (2004) conducted a study to determine the effect of various herbal (pestoban and pestomar) and chemical (fenvalerate and deltamethrin) acarisides on the biological activities of female ticks engorged on cattle and found higher concentration of acarisides stopped egg production while lower concentration reduced egg production.

Fernandes *et al.* (2005) evaluated a larvicidal potential of a crude ethanol extract (CEE) of soupberrt *Sapindus saponaria* stem peel in cattle tick *B. microplus* and lethal concentration valued of 1258 ppm (LC50) and 6360 ppm (LC50) were obtained.

Ghosh *et al.* (2007) evaluated the alcoholic extracts of Sitaphal (*Annona squamosa) and Neem (Azadirachta indica)* for their acaricidal property against different life stages of *H. annatolocum* and *B. microplus* and the initial results were highly encouraging.

Bagherwal *et al.* (2008) studied the efficacy and safety of crude extract of *Annona squamosa linn* (custard apple) containing active ingredient iso-squamocin against naturally occurring *sarcoptic* mange in dogs. Ten applications on alternate days led to complete recovery.

Kumar *et al.* (2008) made effective the solvent selection for plant extraction during in vitro ectoparasidal experiments.

Pirali-Kheirabadi *et al.* (2009) found in vitro acarisidal effect of *Pelargonium roseum* and *Eucalyptus globulus* essential oils against adult stage of *Rhipicephalus* (*Boophilus*) *annulatus* .

Silva *et al.* (2009) evaluated Toxicity of *Piper aduncum* L. (Piperales: Piperaceae) from the Amazon forest for the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* .

Landau *et al.* (2009) used Neem-tree (*Azadirachta indica* Juss.) extract as a feed additive against the American dog tick (*Dermacentor variabilis*) and sheep (*Ovis aries*).

Zorloni *et al.* (2010) reported that *Calpurnia aurea* extracts are used in southern Ethiopia to protect stock against ticks.

Sousa *et al.* (2011) studied Potential synergistic effect of *Melia azedarach* fruit extract and *Beauveria bassiana* in the control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in cattle infestations.

The crude ethanolic extract of aerial parts of *Leucas aspera* was tested for its acarisidal properties against *Rhipicephalus (Boophilus) annulatus by* Ravindran *et al.* in 2011.

Juliet *et al.* (2012) found *Jatropha curcas* (Linn) leaf extract to be a possible alternative for population control of *Rhipicephalus (Boophilus) annulatus*.

Zaman *et al.* (2012) evaluated the anti-tick efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds using adult immersion test, larval packet test and ear bag method against *Rhipicephalus* (*Boophilus*) *microplus.*

Rajakumar and Rahuman (2012) showed Acaricidal activity of aqueous extract and synthesized silver nanoparticles from *Manilkara zapota* against *Rhipicephalus* (*Boophilus*) *microplus*.

Santhoshkumar *et al.* (2012) evaluated stem aqueous extract and synthesized silver nanoparticles using *Cissus quadrangularis* against *Hippobosca maculata* and *Rhipicephalus* (*Boophilus*) *microplus*.

Sindhu *et al.* (2012) reported a new bioassay “syringe test” (modified larval immersion test) for *in vitro* evaluation of acaricidal activity of crude plant extracts against *Rhipicephalus microplus.*

Ghosh *et al.* (2013) found acarisidal properties of *Ricinus communis* leaf extracts against organophosphate and pyrethroids resistant *Rhipicephalus* (*Boophilus*) *microplus.*

Politi *et al.* (2015) showed the action of ethanolic extract from aerial parts of *Tagetes patula* L. (Asteraceae) on hatchability and embryogenesis of *Rhipicephalus sanguineus* eggs.

MATERIALS AND METHODS

The study entitled "In-vitro evaluation of acaricidal properties of *Cymbopogon citrates* & *Nicotiana tabacum* against *Rhipicephalus spp.* collected from cattle and pet dogs" was undertaken in the Department of Medicine, College of Veterinary Science and Animal Husbandry and Department of Zoology, College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar during the period from December 2014 to June 2015.

3.1 Collection of adult female ticks from cattle and pet dogs:

Adult female ticks were collected during morning hours from the cattle of different dairy farms located in the villages near and within Bhubaneswar as well as from different pet dogs visited the clinic of College of Veterinary Science and Animal Husbandry for their treatment. They were kept in transparent plastic tubes with small numerous openings for their ventilation. Small pieces of blotting paper dipped in water were kept inside those tubes and were carried to the laboratory of the Department of Epidemiology Preventive Medicine for in vitro testing.

3.2 Identification of adult female ticks:

Ticks brought for in-vitro testing were identified in the Department of Parasitology, College of Veterinary Science and Animal Husbandry, Bhubaneswar, basing on the characteristics of anal groove, coxa-1, coxa-4, pedipalp, basis-capitulum, presence or absence of festoons, adannal shields and designs of the colours present on the scutum. The ticks were identified up to the genus as per the description of Soulbsy (1982).

3.3Preparation of plants and acaricidal solution:

The following plant materials were collected for the in vitro treatment of the tick.

Table 1. Particulars of plant and plant extract collected:

Sl. No.	Local name	Botanical name	Source
1	Lemon grass	*Cymbopogon citratus*	Laboratory of the Department of Epidemiology Preventive Medicine
2	Tambaku/ Tamakhu (Tobacco)	*Nicotiana tabacum*	Local market

3.3.1 Lemon grass oil: The lemon grass oil was previously prepared from freshly collected leaves by steam distillation method using Clvinjer's apparatus. To 100 ml of distilled water calculated amount of lemon grass oil was added to obtain definite concentration of the solution. The same amount of emulsifier named Tween80 (Polyoxyethylene sorbitan mono-oleate) as lemon grass oil was also added to the solution with the help of a micropipette.

3.3.2 Tobacco: Fifty grams of dry leaves of *Nicotiana tabacum* (Tobacco) were ground to powder using motor and pestle. To obtain different concentrations of solutions, different amounts of powder were added to 100 ml of distilled water and kept overnight.

3.3.3 Chemicals used for in vitro trial:

Information regarding the use of medicines for treatment of tick infestation was collected from the Department of Epidemiology Preventive Medicine.

Table 2. Particulars of chemical used:

Name	Butox
Chemical Composition	Deltamethrin 1.25%
Manufacturer	Samrudh Packaging Pvt. Ltd.
Marketed by	Intervet India Pvt. Ltd.

Preparation of chemicals: To obtain different concentrations of medicine, different dosages of deltamethrin were added to 100 ml of distilled water with the help of a micropipette.

3.4Adult Emulsion Test (AET):

To determine the efficacy of acaricides on female live ticks AET was followed.

3.4.1 AET for cattle ticks: To study the acaricidal property, different experimental sets of petridishes were made ready. In each set four petridishes were used for treatment. Out of these four, three petridishes contained required concentrations of herbal extracts or medicines and one petridish contained equal amount of water. However, for lemon grass oil another petridish with water and tween80 emulsifier in the same quantity as acaricide was also used. Hence, the number of petridishes used in a set for lemon grass essential oil was five. Then, cattle ticks were put in each dish, each containing 10 ticks. The solutions were added to the petridishes up to a height so that the ticks were dipped in it. The entire set of preparation was left for one hour. Then the ticks were transferred to the blotting paper in order to dry the solutions that were on them. The movements of the ticks were observed and the numbers of the paralyzed ticks were recorded. The ticks were left in the same condition with a porous covering for 24 hour. The number of paralyzed or dead ticks within 24 hour was recorded.

Fig 1. Oil and raw form of lemon grass

Fig 2. Powder prepared from tobacco leaves

Fig 3. Deltamethrin or Butox in the container

3.4.2 AET for dog ticks: The dog ticks were collected and were subjected to Adult Emulsion Test following the procedure described above for the cattle ticks. The numbers of ticks subjected to the treatment were the same as that of the cattle ticks so also the concentrations of the herbal and chemical preparations.

3.4.3 Evaluation of efficacy: Efficacy of products i.e. deltamethrin, lemon grass oil, tobacco against the ticks on the basis of their paralytic effect. The following formula was used to ascertain the percentage of efficacy.

$$\% \text{ of efficacy} = \frac{(A-B)*100}{C}$$

Where A= Number of ticks died in test solution

B=Number of ticks died in water

C= Total number of ticks

RESULTS

4.1 Collection of female ticks from cattle and dogs:

More than fifty ticks were collected from cattle of urban rural and areas (Fig. 4) and from pet dogs (Fig. 7) at a time for each set of experiment. The female ticks were identified basing on the characteristics of anal groove, coxa-1, coxa-4, pedipalp, basis-capitulum, presence or absence of festoons, adannal shields, designs of the colors present on the scutum and the ovipositor.

4.2 Effect of plant products and chemicals on cattle ticks:

Lemon grass oil, tobacco powder and deltamethrin solutions were applied in order to check their effects on cattle ticks collected from urban and rural areas. The effects of the solutions were paralytic as well as lethal during the period of observation and were noted down in the observation tables (Table No.3, 4, 5, 6, 7and 8)

4.2.1 Effect of lemon grass oil, tobacco and deltamethrin on cattle ticks collected from urban areas:

Effects of lemon grass oil, Tobacco and deltamethrin on cattle ticks collected from urban areas have been depicted in table 3. The effective concentration of deltamethrin (0.3 ml/100ml water) revealed that within one hour of exposure out of ten ticks, only two (20%) and within 12 hours all (96.6%) were paralyzed. This concentration was compared with the same concentration of lemon grass oil (0.3ml/100ml water) and tobacco leaves (300mg/ 100ml water) solution. The effect of lemon grass oil on ticks showed that only three (26.6%) and ten (96.6%) were paralyzed within 1 hour and 24 hours after treatment respectively. No ticks (0%) were paralyzed on treatment with tobacco leaves with in 1 hour, but out of ten ticks, only one tick (10%) was paralyzed within 24 hours of exposure. In control no ticks died with 0% (water) effectiveness. As tween80 was mixed with lemon grass oil solution, it was also added in the same concentration as that of lemon grass oil in water to check its paralytic effect. However, it did not show any effect on ticks within 1 hour and 24 hours of treatment.

Effect of lemon grass oil and tobacco on cattle ticks collected from urban areas:

The concentrations of lemon grass oil and tobacco in the solution were changed in order to check the lethal effect (Fig. 6) and the concentrations were accordingly increased and decreased. The ticks were treated with them and results were recorded (Table no.4 and 5).

4.2.1.1 Effect of lemon grass oil on ticks:

Ten ticks were treated with 0.1 ml, 0.2ml, 0.3ml and 0.5ml concentration of lemon grass oil. Out of ten ticks for each concentration one (10%), two (16.6%), three (26.6%) and seven (70%) ticks were paralyzed within 1 hour and nine (90 %), nine (93.3%), ten (96.6%) and ten (100%) ticks were paralyzed within 24 hours of exposure (Table. 4 & Fig.6).

4.2.1.2 Effect of tobacco on ticks:

100mg /100ml, 200mg/100ml, 300mg/100ml and 600mg/100ml of solution of powdered tobacco leaves were applied on the ticks in order to check the paralytic effect. Out of ten ticks no ticks died within 1 hour for 100mg, 200mg and 300mg of concentrations. However, no ticks also got paralyzed for 100mg concentration within 24 hours. But one (6.6%) and one (10%) ticks were paralyzed out of ten ticks for 200mg and 300mg concentration within 24 hours of treatment and one (10%) and two (20%) ticks were paralyzed within 1 hour and 24 hours for 600mg concentration. (Table.5 and Fig.6)

4.2.2 Effect of lemon grass oil, tobacco and deltamethrin on cattle ticks collected from rural areas:

Ticks collected from rural areas were exposed to the lemon grass oil, tobacco, deltamethrin having paralytic effects. The effects were presented in table-6. Out of ten ticks immersed in 0.6ml of lemon grass oil solution, three (33.3%) ticks and nine (90%) ticks were found to be paralyzed within 1hour and 24 hours of exposure respectively. At the concentration of 600mg of tobacco in 100ml of water, two (16.6%) ticks and six (63.3%) ticks out of ten ticks showed paralytic effect within 1 hour and 24 hours of exposure respectively. The 0.6ml concentration of deltamethrin showed that six (60%) ticks and all ticks (96.6%) ere paralyzed within 1 hour and 24 hours of exposure respectively. However, no ticks (0%) died in control. The effect of tween80 was also tested taking same concentration as that of lemon grass oil in solution. But, no ticks (0%) also got paralyzed here even after 24 hours of exposure.

Effect of lemon grass oil and tobacco on cattle ticks collected from rural areas:

The ticks of the cattle collected from rural areas were also treated with the varying concentrations of lemon grass oil and tobacco in order to determine the lethal concentration. The ticks were treated with them and the results were presented in (Table no.7 and 8).

4.2.2.1 Effect of lemon grass oil on ticks:

Effects of different concentrations of lemon grass oil were presented in table-7. 0.1ml/100ml, 0.2ml/100ml, 0.3ml/100ml, 0.4ml/100ml, 0.5ml/100ml and 0.6ml/100ml concentrations of lemon grass showed that out of ten ticks 0(0%), one (10%), two (16.6%), two (16.6%), two (16.6%), two (23.3%), three (33.3%) ticks were paralyzed within 1 hour of exposure and four (40%), six (60%), seven (66.6%), seven (70%), eight (83.3%) and nine (90%) were found dead within 24 hours of treatment respectively.

4.2.2.2 Effect of tobacco on ticks:

Different concentrations of powdered tobacco were applied on ticks and the effects were noted in table-8. No ticks (0%) were paralyzed in 100mg/100ml and 200 mg/100ml of solutions within 1 hour and 24 hours. No ticks (0%) also died in 1 hour of exposure in 300mg/100ml concentration, but one (10%) tick died out of ten ticks within 24 hours. For 600mg/100ml, 1500mg/100ml and 2000mg/100ml concentrations, two (16.6%), two (33.3%), five (46.6%) were died out of ten ticks within 1 hour and six (63.3%), seven (73.3%), eight (83.3%) ticks were killed within 24 hours of exposure.

4.3 Effect of plant products and chemicals on dog ticks:

The ticks collected from different pet dogs were exposed to different concentrations of lemon grass oil, tobacco and deltamethrin for 1 hour and effects were observed within 1 hour and 24 hours (Table no.9, 10 and 11).

4.3.1 Effect of lemon grass oil, tobacco and deltamethrin on dog ticks:

A concentration having a considerable paralytic effect was selected for all the herbal products and chemical. Their effects during 1 hour and 24 hours were noted in table-9. 0.5ml/100ml, 1500ml/100ml, 0.5ml/100ml concentrations of lemon grass oil, tobacco and deltamethrin showed that eight (83.3%), four (46.6%), nine (86.6%) ticks and ten (100%), seven (73.3%), nine (96.6%) ticks out of ten ticks were paralyzed within 1 hour and 24 hours respectively for subsequent concentrations. However, tween80 at a concentration of 0.5ml/100ml of water showed no (0%) paralytic effects within 1 hour and 24 hours.

4.3.2Effect of lemon grass oil and tobacco on dog ticks collected:

As done in the treatment of cattle ticks of urban area, the ticks collected from different pet dogs were also treated with the varying concentrations of lemon grass oil and tobacco in order to know the lethal concentration. The concentrations were increased and decreased accordingly. The ticks were treated with them and the results were recorded. (Table.9)

4.3.2.1 Effect of lemon grass oil on ticks:

The effects of different concentrations of lemon grass oil on dog ticks have been presented in table 10. At concentrations of 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml and 1ml of lemon grass oil, four (36.6%), four (43.3%), six (56.6%), eight (76.6%), eight (83.3%) and nine (86.6%) ticks were paralyzed out of ten ticks within 1 hour respectively. Similarly out of ten ticks, four (36.6%), four (43.3%), six (56.6%), eight (80%), ten (100%), ten (100%) ticks were paralyzed within 24 hours at concentrations of 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml and 1ml of lemon grass oil respectively.

4.3.2.2 Effect of tobacco on ticks:

At concentrations of 100mg, 200mg, 300mg, 600mg, 1000mg, 1500mg, 2000mg of powdered tobacco per 100ml of water, one (10%), one (13.3%), two (16.6%), three (33.3%), four (40%), ten (96.6%) and ten (100%) ticks were paralyzed out of ten ticks within 24 hours respectively. However, paralytic effect on one (13.3%), two (23%), three (26.6%), three (30%) ticks out of ten ticks was observed for 600mg, 1000mg, 1500mg, 200mg concentrations respectively within 1 hour (Table.11).

SUMMERY AND CONCLUSION

Tick infection is a common problem in dairy & in pet animals like dogs. *Rhipicephalus spp*. cause economic loss in terms of milk production in cattle, loss of body weight, loss of body hair and acts as vectors of several diseases like anaplasmosis, babesiosis, ehlichosis etc. Effective therapeutic and preventive measures are available through administration of acaricides in the farm premises and on pet dogs. But mounting information on development of drug resistance & hazards of chemical acaricides has become a matter of concern. This triggered to design a study to find out alternative sustainable control strategies using herbal preparations.

Adult ticks were collected from urban, rural areas and also from different pet dogs visiting the central clinic of college of Veterinary Science and Animal Husbandry. Adult female ticks were identified up to genus.

Two plant materials i.e. lemon grass oil and tobacco leaves were selected from the list of medicinal plants having acaricidal property. Deltamethrin, a chemical acaricide, presently used in firm premises was included in the study. Test materials were added to ticks at a rate to achieve the final working concentration. The ticks were immersed for 1 hour and the effects on ticks were recorded to ascertain the inhibition or lethal action. Effects of the above test materials were evaluated within 1 hour and 24 hours of exposure. The paralytic effects in the above mentioned 3 categories of ticks were compared with that of untreated control.

Results of the present study showed some differences in the paralytic effect on addition of different concentration of lemon grass oil, tobacco leaves and deltamethrin. When compared with untreated control, only lemon grass oil and deltamethrin were highly effective in case of cattle and dog ticks. In case of cattle ticks collected from urban areas, both deltamethrin and lemon grass oil had the same acaricidal efficacy but the effect of tobacco was negligible. But in case of ticks collected from rural areas, deltamethrin was more effective than lemon grass oil followed by tobacco. In case of dog ticks, lemon grass oil was highly effective followed by tobacco leaves and deltamethrin. However, ticks treated in control did not show any paralytic or lethal effect.

Observation with respect to acaricidal property reveals that lemon grass oil at a concentrations of 0.3%, 0.6% and 0.5% produce superior paralytic effect on ticks collected from cattle of urban, rural areas and pet dogs respectively and the effect was faster than of tobacco leaves and deltamethrin. All ticks become paralysed within 45 minutes of treatment with lemon grass oil, where as all ticks became immobile within 24 hour in deltamethrin and tobacco leaves.

It was concluded that the effect of lemon grass oil was highest on the ticks. Besides antibacterial and insect repellent properties, that herbal product exhibited in-vitro acaricidal action. Thus lemon grass oil can be used as an acaricide in dairy farms and for pet dogs to resist tick infection. However, due to its higher market price one cannot use it frequently. Comparison between deltamethrin and tobacco leaves indicate that deltamethrin had some adverse effects on the host animal though it acted better than tobacco. Though tobacco is less effective, it is easily available in market. When lemon grass oil vs. deltamethrin is debated on the issue relating their acaricidal properties, the former is considered as more ideal for control of tick infection. Further work may reveal additional advantages of such plants materials and also identification of the ticks up to the species level.

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